Reviews

Molecular pathogenesis of oral squamous carcinoma

H K Williams

Abstract

Oral squamous carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumour suppressor genes. This can result in increased production of growth factors or numbers of cell surface receptors, enhanced intracellular messenger signalling, and/or increased production of transcription factors. In combination with the loss of tumour suppressor activity, this leads to a cell phenotype capable of increased cell proliferation, with loss of cell cohesion, and the ability to infiltrate local tissue and spread to distant sites. Recent advances in the understanding of the molecular control of these various pathways will allow more accurate diagnosis and assessment of prognosis, and might lead the way for more novel approaches to treatment and prevention.

(*J Clin Pathol: Mol Pathol* 2000;**53**:165–172)

Keywords: oral squamous carcinoma; oncogenes; tumour suppressor genes

Department of Oral Pathology, Birmingham Dental Hospital and School, St Chad's Queensway, Birmingham, B4 6NN, UK
H K Williams

Correspondence to: Dr Williams

email: h.k.williams.den@Bham.ac.uk

Accepted for publication 25 May 2000

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy, although it only accounts for 2% of all cancers in Western populations.[1] However, the incidence of head and neck cancer, in particular tumours of the larynx and oral cavity, are increasing in developed countries, with the increase of risk being seen in younger people, particularly young women.[2] Despite therapeutic changes survival remains poor, mainly because of the increased risk of developing a second malignancy, which can be a second primary carcinoma.[3 4]

Cytogenetics of oral cancer

Oral carcinogenesis is a multistep process in which genetic events lead to the disruption of the normal regulatory pathways that control basic cellular functions including cell division, differentiation, and cell death. Several studies have shown that there is a genetic component in the development of carcinoma. These include reports of the occurrence of familial aggregations of cancer, including oral cancer, with carcinomas developing at a younger age.[5 6] An increased risk of oral squamous carcinoma has also been detected among relatives outside the immediate family of patients with squamous cell carcinoma. Several studies have demonstrated a higher risk for multiple primary tumours in patients from families where more than one relative has had squamous carcinoma of the upper aerodigestive tract.[7–9]

Whether patients develop single site oral cancer or multiple site oral cancer, much evidence has accumulated to suggest that multiple genetic events lead to oral cancer, with around six to 10 genetic events believed to result in oral carcinogenesis. However, the importance of both the known gene alterations and as yet unidentified oncogenes and tumour suppressor genes is still not fully understood.[10 11] Genetic alterations known to occur during carcinogenesis including point mutations, amplifications, rearrangements, and deletions. Point mutations (single base changes) can lead to overactivity or inactivity of gene products. These are common in genes such as K-ras and p53. Amplifications and rearrangements have frequently been reported in malignant neoplasms, with both amplification and rearrangement affecting excitatory pathway genes, whereas rearrangement can also inactivate inhibitory pathway genes.[11]

Several studies have identified specific genetic alterations in oral carcinomas and in premalignant lesions of the oral cavity. Recently, using comparative genomic hybridisation on 50 primary head and neck carcinomas, Bockmuhl and colleagues reported deletions of chromosome 3p, 5q, and 9p with 3q gain in well differentiated tumours, whereas in poorly differentiated tumours deletions of 4q, 8p, 11q, 13q, 18q, and 21q and gains in 1p, 11q, 13, 19, and 22q were identified, thus suggesting an association with tumour progression.[12] With the development of molecular techniques, such as microsatellite assays and restriction fragment length polymorphism, it has been shown that allelic imbalance of chromosomal 9p is the most common chromosomal arm loss in head and neck squamous cell carcinoma.

Loss of heterozygosity (LOH) was reported at 9p21–p22 in 72% of tumours.[13] More recently, Partridge and colleagues identified five areas in the region of allelic imbalance at chromosome 3p that might harbour tumour suppressor genes, along with two areas at 8p and 9p, respectively.[14] These authors also

identified significantly greater allelic imbalance in patients with TNM stage 4 disease compared with stages 1–3. Allelic imbalance at one or more loci within 3p24–26, 3p21, 3p13, and 9p21 was associated with reduced survival, with a 25 fold increase in mortality rate with allelic imbalance at 3p24–26, 3p21, and 9p21 compared with patients retaining heterozygosity at these loci.[15]

Allelic loss of 3p and 9p and other regions containing tumour suppressor genes has also been reported in precursor lesions of oral cancer showing varying degrees of dysplasia compared with normal epithelium.[16] Allelic loss or imbalance at p53, DCC (deleted in colon carcinoma), and regions at 3p21.30–22, and 3p12.1–13 were reported, with LOH at DCC shown to occur in areas of dysplasia adjacent to infiltrating carcinoma. This suggested that loss at this locus might be a later event, whereas LOH at 3p and p53 were more frequent in those dysplastic lesions that recurred.[17] These authors also demonstrated that in those dysplastic lesions that recurred, allelic imbalance was present in the initial lesion and that allelic imbalance increased as the carcinoma developed.[17] More recently, these researchers reported allelic loss in 77% of premalignant lesions similar to that found in oral carcinomas, with 55% of cases showing microsatellite instability.[18] These patients carried a 73% probability of developing oral squamous carcinoma within five years; the authors concluded that determining the number of genetic abnormalities in potentially malignant lesions can help identify those patients at risk and who may benefit from chemoprevention, such as retinoic acid derivatives, which may reduce the incidence of transformation of premalignant lesions or help prevent the development of a second primary tumour in the upper digestive tract.[18 19]

Chromosome breakpoints are frequently seen in centromeric regions of chromosomes 1, 3, 8, 14, 15, 1p22, 11q13, and 19p13. Because genes bcl-1, int-2, and hst-1 have been mapped to 11q13 and n-ras to 11q13, it has been suggested that activation of these oncogenes is the result of these cytogenic alterations.[11 20]

Approximately two thirds of all head and neck cancers contain a deleted region in chromosome 9p21–22. The cyclin dependent kinase inhibitor 2/multiple tumour suppressor gene 1 (CDKN2/MTSI) has been mapped to this chromosome region, and inactivation of its protein product $p16^{INK4}$ by mutation and deletion has been found in 10% and 33% of head and neck squamous carcinomas, respectively, along with frequent inactivation of p16 in oral premalignant lesions. This suggests an important role for this gene in the early stages of oral carcinogenesis.[21] Cyclins, cyclin dependant kinases (CDKs), and cyclin dependent kinase inhibitors regulate progress through key transitions in the cell cycle. $p16^{INK4}$ binds to and inhibits phosphorylation of pRb by the cyclin dependent kinases CDK4 and CDK6.[22 23]

Other proteins that regulate crucial checkpoints in the cell cycle, and which are important contributors to increased cell proliferation, include cyclin D, E, and A, which regulate the G1 to S phase transition, and cyclin B, which regulates the G2 to M transition.[24 25]

The cyclin D1 gene is frequently overexpressed in oral cancers as a result of amplification of the 11q13 region.[26] Overexpression of cyclin A has been reported in oral carcinomas, with the increase in expression being associated with tumour grade.[27] Cyclin B was also reported to be overexpressed, with increased cytoplasmic staining compared with nuclear staining in normal cells. Cyclin B1 binds to protein kinase $p34^{cdc2}$ in the cytoplasm of the dividing cells, and the complex is transported to the nucleus at the G2 to M transition.[22] This suggests that frequent abnormalities in cyclin B/$p34^{cdc2}$ kinetics in oral carcinomas lead to deregulation of the G2 to M transition.

Oncogenes

Oncogenes are altered growth promoting regulatory genes that govern the cells' signal transduction pathways,[28] and mutation of these genes leads to either overproduction or increased function of the excitatory proteins.[28] Although oncogenes alone are not sufficient to transform epithelial cells, they appear to be important initiators of the process, and are known to cause cellular changes through mutation of only one gene copy.[10]

Several oncogenes have been implicated in oral carcinogenesis. Aberrant expression of the proto-oncogene epidermal growth factor receptor (EGFR/c-erb 1), members of the ras gene family, c-myc, int-2, hst-1, PRAD-1, and bcl-1 is believed to contribute towards cancer development.[11]

Deregulation of growth factors occurs during oral carcinogenesis through increased production and autocrine stimulation.[29 30] Aberrant expression of transforming growth factor α (TGF-α) is reported to occur early in oral carcinogenesis, first in hyperplastic epithelium, and later in the carcinoma within the inflammatory cell infiltrate, especially the eosinophils, surrounding the infiltrating epithelium. TGF-α stimulates cell proliferation by binding to EGFR in an autocrine and paracrine fashion.[31] TGF-α is believed to stimulate angiogenesis and has been reported to be found in "normal" oral mucosa in patients who subsequently develop a second primary carcinoma.[32 33]

EGFR, the biological receptor of EGF and TGF-α is frequently overexpressed in oral cancers,[30 34] and this was found to be the result of EGFR gene amplification in 30% of oral cancers.[35] It has been suggested that overexpression of the EGF receptor is often accompanied by the production of its ligands, TGF-α and EGF. The interaction of the receptor and its ligands initiates a cascade of events, translating extracellular signals through the cell membrane and triggering intrinsic tyrosine kinase activity. Mutations of genes encoding growth factor receptors can result in an increased number of receptors, or the production of a continuous ligand independent mitogenic signal. Gene amplification and increased numbers of EGF receptors in oral

cancers are associated with the degree of differentation and aggressiveness of the tumours.[33 36] Oral squamous carcinomas overexpressing EGFR have been shown to exhibit a greater response to chemotherapy, compared with EGFR negative tumours. This is presumably because of the higher proliferative activity in the tumours overexpressing EGFR, leading to a higher sensitivity to cytotoxic drugs,[37] and current data suggest that the therapeutic application of antibodies directed against EGF receptors might be useful in the treatment of premalignant and malignant lesions.[38]

During oral carcinogenisis, intracellular messengers might also be intrinsically activated, thereby overriding the necessity for ligand–receptor regulated signals.[39] Among the genes involved in intracellular signalling pathways, members of the ras family have been examined in oral cancers. H-ras, K-ras, and N-ras all encode the protein p21, which is located on the cytoplasmic membrane of the cell membrane, and which transmits mitogenic signals by binding GTP. The mitogenic signal is terminated by the conversion of GTP to GDP by hydrolysis, but when the ras oncogene is mutated this conversion can be prevented, thus leading to continuous stimulation.[11]

Some studies have indicated that members of the ras oncogene family are overexpressed in oral cancers.[40–42] Although loss of control of N-ras might be an early step in carcinogenisis in oral cancers, with increased expression occurring early in dysplastic lesions, ras mutations are uncommon in the progression of oral cancers in the Western world, occurring in less than 5% of all cases.[41 42] In contrast, 55% of lip cancers have H-ras mutation[43] and H-ras mutation occurs in 35% of oral cancers in the Asian population, where it is especially associated with betel nut chewing.[44 45]

Transcription factors that activate other genes are also activated in oral cancer. The functional activity of many of these proteins is regulated by receptor activated second messenger pathways, and neutralisation of these genes could result in a cell cycle block, preventing mitogenic and differentiation responses to growth factors. Among these genes c-myc, which helps regulate cell proliferation, is frequently overexpressed in oral cancers as a result of gene amplification.[46 47] Overexpression is frequently associated with poorly differentiated tumours,[11] although more recently c-myc has been shown to be overexpressed in moderate and well differentiated oral carcinomas, in which cell proliferation far outweighed the number of apoptotic cells present (HK Williams *et al*, unpublished data, 1999). c-Myc induces both cell proliferation and apoptosis. c-Myc requires p53 to induce apoptosis and the retinoblastoma tumour suppressor gene Rb-1 nuclear protein pR6 interacts with the c-myc gene, preventing its transcription, and thus inhibiting cell proliferation.[48] However, on phosphorylation of pR6, c-Myc is increased and cell proliferation proceeds.[49] In this regard, it is interesting that we found pR6, c-myc, and p53 to be expressed in all oral carcinomas, irrespective of differentation. However, further studies are needed to determine the genetic status of these oncogenes and tumour suppressor genes,[4] and to measure the concentrations of the proteins, to determine possible controlling pathways in the development of these oral cancers (HK Williams *et al*, unpublished results, 1999).

The PRAD-1 gene located on 11q13 encodes cyclin D, which together with the Rb gene product controls the G1 to S transition of the cell cycle. The PRAD-1 gene is amplified in 30–50% of head and neck cancers. Amplification of PRAD-1 is correlated with cytological grade, infiltrative growth pattern, and metastases.[50 51]

The hst-1/int-2 gene encodes a protein that is homologous to fibroblast growth factor,[52] and which in oral cancers has been shown to be involved in tumour growth,[53] and to have angiogenic activity.[54] This gene maps to human chromosome 11q13.3, which is coamplified with int-2 in some cancers.[52]. Lese *et al*, in 1995, reported coamplification of the int-2 and hst-1 genes in oral squamous carcinomas, and other authors have suggested that this coamplification in head and neck squamous carcinomas is associated with tumour recurrence and progression of disease.[55 56]

Int-2 amplification has also been described in premalignant lesions adjacent to neoplasia, both in areas of dysplasia and hyperplasia, which suggests that int-2 can be amplified before tumour development.

Tumour suppressor genes

Oncogenes alone are not sufficient to cause oral cancer and appear to be initiators of the process. The crucial event in the transformation of a premalignant cell to a malignant cell is inactivation of cellular negative regulators—tumour suppressor genes—and is regarded to be a major event leading to the development of malignancy. Tumour suppressor genes are most often inactivated by point mutations, deletions, and rearrangements in both gene copies.[57–59]

There has been much research on the tumour suppressor gene p53. The p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage, and also induces apoptosis. These functions are achieved by the ability of p53 to modulate the expression of several genes.[59] The p53 protein transcriptionally activates the production of the p21 protein, encoded by the WAF1/CIP gene, p21 being an inhibitor of cyclin and cyclin dependant kinase complexes.[60] p21 transcription is activated by wild-type p53 but not mutant p53.[61] However, WAF1/CIP expression is also induced by p53 independent pathways such as growth factors, including platelet derived growth factor, fibroblast growth factor, and transforming growth factor β.[62] Wild-type p53 has a very short half life (four to five minutes),[63] whereas mutant forms of protein are more stable, with a six hour half life.[64]

Mutation of p53 occurs either as a point mutation, which results in a structurally altered protein that sequesters the wild-type protein, thereby inactivating its suppressor activity, or

by deletion, which leads to a reduction or loss of p53 expression and protein function. The tumour suppressor gene p53 is known to be mutated in approximately 70% of adult solid tumours.[65]

p53 has been shown to be functionally inactivated in oral tumours, and restoration of p53 in oral cancer lines and tumours induced in animal models has been shown to reverse the malignant phenotype.[66] Smoking and tobacco use have been associated with the mutation of p53 in head and neck cancers,[67 68] and in lung cancer.[69]

Among several diverse genes that contain wild-type p53 binding sequences is mdm2 (the human homologue to the murine double minute 2 oncogene). This gene encodes a p53 binding protein that forms an autoregulatory feedback loop with the normal p53 protein. The gene has been found to be amplified in many human malignancies, thus abolishing the antiproliferative function of p53.[70] Until recently, information on the association between p21, p53, and mdm2 in oral cancer has been scanty; however, a recent study has shown that this putative tumour suppressor gene p21/WAF1 is overexpressed in oral squamous carcinomas. This overexpression is related to the cell proliferation index and mdm2 expression, but is independent of p53 protein alteration.[71] Other studies have demonstrated p21 in premalignant and malignant oral lesions, suggesting that alteration in p21 expression is an early event in oral carcinogenesis, with increased expression occurring via p53 dependent and independent pathways.[72] Overexpression of p21 alone, however, appears insufficient to induce tumour progression.[73] p21 gene mutation has not been found in oral cancers and is infrequent in other cancers.[71 74]

In general, tumour suppressor genes are thought to act recessively so that both copies of the gene must be inactivated for malignancy to occur. LOH and p53 mutations have been reported in several tumours—for example, in breast and prostate cancer,[75 76] but a lack of concordance has been found in oral cancers. In these cases, it has been argued that mutant p53 overrides the activity of the wild-type protein.[77]

There is also controversy about the relation between p53 mutation and detection of the p53 protein by immunocytochemistry. Some authors have suggested a high correlation between p53 expression and point missense mutation,[78] whereas others have reported some discrepancy in oral cancer, with lack of expression demonstrated by immunocytochemistry having been attributed to insensitive methods of detecting p53 mutation, or the existence of truncating mutations that result in the absence of protein.[79–81]

However, stabilisation of p53 and detection by immunocytochemistry might not necessarily be the result of mutation. In Li-Fraumeni syndrome, p53 is mutant but the protein is unstable, like the wild-type p53 protein, which suggests that some other event may be necessary for stability, and that stability of p53 is not intrinsic to the mutant p53 structure, but might vary in different cell backgrounds.[81 82]

The relation described earlier between p53 and mdm2 appears to be important because when normal p53 is bound to mdm2 it is targeted for destruction by the ubiquitin dependent pathway.[83] However, it appears that mutant p53 fails to stimulate transcription of mdm2 and therefore mutant p53 is not degraded.[84] Alternatively, mutated or altered expression of the mdm2 gene might be responsible for the stabilisation of apparent wild-type p53 in tumours without p53 mutation.[81]

Recently, Partridge and colleagues endeavoured to shed light on the mechanisms that permit detection of mdm2 in these p53 mutation negative cases of oral cancer. They examined 45 cases of oral squamous carcinoma for p53 mutation, allelic imbalance of the p53 protein, and mdm2 expression. Twenty three per cent of cases showed an allelic imbalance and 33% of oral tumours showed p53 mutation; there was low concordance between allelic imbalance and mutations, suggesting that normal and mutant p53 may coexist in the same tumour. However, oncogenic human papillomavirus (HPV) has been reported to be present in 10–42% of tumours without p53 mutation.[85] Thus, if the E6 protein forms complexes with wild-type p53 and promotes p53 degradation,[86] this could account for the lack of concordance between p53 mutation frequency and LOH in this study.[81 86]

Mdm2 values were also consistently low in this study, irrespective of whether p53 was mutated or not, which suggests that factors other than p53 regulate mdm2. However, although the mdm2 status of oral tumours is not known, these authors suggested that non-functional wild-type p53 in some oral cancers might be the result of the inability of mdm2 to degrade p53, either as a consequence of mutation or as a result of interaction with other regulatory molecules.[81]

Other tumour suppressor genes include doc-1, the retinoblastoma gene, and APC. The doc-1 gene is mutated in malignant oral keratinocytes, leading to a reduction of expression and protein function.[87] The precise function of the Doc-1 protein in oral carcinogenisis is unclear, but it is very similar to a gene product induced in mouse fibroblasts by tumour necrosis factor α (TNF-α). Normally, TNF-α decreases proliferation and increases differentiation, and has been shown in oral squamous cell carcinoma cell lines to be responsible, either alone or in combination with interferons α or γ, for antiproliferative activity.[11]

The retinoblastoma tumour suppressor gene regulates the cell cycle by the hypophosphorylated protein pRb preventing cells from transition across the G1 checkpoint, by sequestering transcription factors such as E2F, which activate S phase genes. Although phosphorylation of pRb cancels this growth suppressive function, $p16^{INK4}$ inhibits phosphorylation of pRB via the CDKs. pRb also suppresses apoptosis, except after DNA damage, when apoptosis is induced. In its hypophosphorylated form, the Rb protein binds to the c-myc gene, blocking cell progression. However, on phosphorylation, E2F is released, the cells pass the G1 to S

boundary, transcription factors such as c-Myc are increased, and cell proliferation proceeds.[49 88]

In some reports, lack of pR6 expression has been observed in 66% of oral squamous carcinomas and 64% of premalignant lesions. p16 expression is absent in 63% of oral squamous carcinomas and in 59% of premalignant lesions. Alteration in pRb/p16 expression correlated with heavy consumption of betel and tobacco, suggesting that alteration in the p16/pR6 pathway is an early event in oral tumorigenesis and might be involved in the development of betel and tobacco related malignancies.[23] This is in contrast to our recent observations, which show that pRb6 is strongly expressed in oral squamous carcinomas, irrespective of differentiation (HK Williams *et al*, unpublished data, 1999). Further studies are clearly necessary to elucidate its role in oral carcinogenesis .

LOH with mutation of the adenomatous polyposis coli (APC) tumour suppressor gene has not been detected frequently in oral cancers, although one study[89] described LOH in 25% of oral cancers and Huang *et al* suggested a higher number, with 53.8% showing LOH.[90] Recently, the function of APC has become better understood, especially in relation to cell adhesion molecules.

Cell adhesion molecules

Cell surface molecules might also be important in inhibiting oral keratinocyte proliferation and angiogenesis. E-caderin—a cell adhesion molecule associated both with invasion and metastasis—is downregulated in oral cancers.[91–93]

Recently, using immunocytochemistry and confocal microscopy, we have shown that in primary oral squamous cell carcinomas and concurrent metastatic disease, P-cadherin is consistently upregulated, and localisation of the catenins associated with E-cadherin is altered.[93] Western blot analysis has also confirmed downregulation of γ-catenin, which suggests that the desmosomal proteins may be downregulated (HK Williams *et al*, unpublished data, 1999).

Although the exact genetic events that result in degradation of the E-cadherin–catenin complex in oral carcinogenesis is not understood, mutations of E-cadherin, α-catenin, and β-catenin in other tumours have been described.[94–96] β-Catenin is now widely recognised as an essential element in the Wingless/Wnt signalling cascade.[95] In the absence of Wnt signalling, APC is phosphorylated by the serine kinase, glycogen synthase kinase 3-β (GSK3-β), and then interacts with β-catenin and indirectly with α-catenin, forming an APC–catenin complex. However, Wnt signalling antagonises GSK-3β activity, leading to the cytoplasmic accumulation of β-catenin. This is known to occur where mutant APC is present in colon carcinoma cell lines.[96] Recent evidence also shows that APC controls certain transcriptional activities because loss of APC function in colon carcinoma cells results in cytoplasmic β-catenin transducing Wnt signals by associating with T cell factor (TCF) and lymphoid enhancer factor (LEF). These complexes then pass to the nucleus where they activate Tcf target genes in an uncontrolled manner, which might contribute to colon tumorigenesis.[97] Although few cases of mutant APC have been described in oral carcinoma, further investigations are required to investigate the function of β-catenin in oral cancer.

Recently, it has been shown that APC might indirectly regulate the E-cadherin–catenin complex because in E-cadherin negative colon carcinoma cell lines, β-catenin is preferentially bound to APC. If, however, these cell lines are transfected with E-cadherin, β-catenin redistributes from the APC bound complex to the E-cadherin–catenin complex and is accompanied by growth inhibition and decreased tumorigenicity.[98 99] At present, however, it is unknown whether APC controls the E-cadherin–catenin complex in oral carcinoma.

Other cell adhesion molecules include the cation dependent, heterodimeric family of integrins, which mediate cell–cell and cell–matrix interactions, and play a role in the maintenance of tissue integrity and in the regulation of cell proliferation, growth, differentiation, and migration. Integrin expression is variable between different tumour types but these molecules are implicated in tumour progression and metastasis.

In oral squamous cell carcinomas, there is variable loss or reduced expression of β1 integrins and α6β4, especially in poorly differentiated carcinomas. The localisation and the quantity of the α6 chain has been shown to be altered, with high levels of α6 in contrast to β4, both in premalignant and malignant oral mucosa. This suggests that this might be an early but non-specific marker of oral malignancy, and that abnormal extracellular signals might be involved.[100] Recently, it has been reported that metastatic oral squamous carcinoma cell lines show strong expression of α2–6 integrins compared with non-metastatic cell lines, and the pronounced expression seen in primary oral squamous carcinomas correlates significantly with the mode of tumour invasion and nodal involvement.[101]

The expression of αv is also altered, with αvβ6 being expressed in malignant oral carcinomas. This integrin is not expressed in normal epithelium, suggesting that it might play a role in tumour migration. Reduced expression is seen for αvβ5 in oral cancers in contrast to normal epithelium. This integrin might be important in oral neoplasia because in vitro studies suggest that αv negative malignant cell lines can be reversed after transfection of the integrin.[102 103]

Squamous cell carcinomas of the oral cavity are characterised by their ability to spread locally and regionally, this being associated with a high rate of fatality, with a breach in the basement membrane separating epithelial and mesenchymal compartments being the first step in tumour invasion.[104 105] Not only does the alteration in expression and/or function of several of these cell adhesion molecules result in

tumour infiltration and metastasis, but there is now compelling evidence that urokinase 92 kDa type IV collagenase (MMP-9) is produced by tumour cells, whereas type I collagenase and stromeolysins 2 and 3 are synthesised by stromal cells[105]. However, 72 kDa type IV collagenase (MMP-2), also found in oral cancers, is stimulated by MT-MMP, a cell membrane metalloproteinase. Recent evidence suggests that stromal cells influence the synthesis of urokinase and MMP-9, although the transcriptional requirements and cell signalling pathways remain to be fully elucidated.[104]

Viruses

Many viruses have been associated with oral epithelial dysplasia and squamous carcinoma. There are over 77 types of HPV based upon differences in DNA sequences. Oral epithelial dysplasia, which is a precursor to squamous carcinoma, is infected with HPV, and types 2, 6, 11, 16, 18, 31, 33, 35 have been detected. Moreover 75% contain the high risk HPV types 16 and 18. HPV is also found in 33–50% of oral squamous cell carcinomas and again most of these contain HPV-16 and HPV-18. These viruses contain gene products (E6 and E7) that bind wild-type p53 and Rb proteins and eliminate the ability of these proteins to stimulate DNA repair or apoptosis.[35]

Another virus that has been implicated in malignant transformation is human herpesvirus 6 (HHV-6). HHV-6 has been reported in oral squamous carcinomas but it is unclear whether this finding has any clinical relevance.[35 106] Possible methods of antiviral treatment, including antiviral targeted gene therapy and immunotherapy, may become possible in the near future.

Tumour growth

The growth of cancer is closely dependent on the balance between cell growth and cell death.[107] Fas (CD95/APO-1), a transmembrane protein related to the TNF-R/NGF-R family, has been shown to be part of a major effector pathway involved in the regulation of apoptosis. Fas is activated by its ligand FasL, which results in the induction of apoptosis.[108 109] Recently, it was reported that the Fas receptor is not highly expressed in oral squamous carcinomas although the ligand FasL was high in oral carcinomas, particularly in poorly differentiated carcinomas. However, the functional consequences of these findings are not understood.[110] These workers also showed that apoptosis in oral squamous carcinoma was lower in poorly differentiated carcinomas, but they speculated about whether this was the result of loss of Fas expression or increased anti-apoptotic factors.[110]

The Bcl-2 family of proteins appears to regulate apoptosis via differential homodimerisation and heterodimerisation. Bcl-2–Bax heterodimers are an important anti-apoptotic moiety, whereas Bax–Bax homodimers promote cell death. Few studies have been undertaken to investigate apoptosis in oral cancers, although Jordan and colleagues demonstrated that Bcl-2 was present in poorly differentiated cancers, whereas Bax was present in differentiated oral cancers.[111] In a more recent study in oral cancers from an Asian population, low concentrations of Bax were demonstrated, with a high concentration of Bcl-2, irrespective of tumour differentiation.[112] In this regard, it is interesting that recently we have observed in oral primary and metastatic squamous carcinomas an increased expression of $Bclx_s$, Bik, and Bax—proteins that stimulate apoptosis—in contrast to those that inhibit apoptosis, including Bcl_2, Mcl-l, and Bcl-x (HK Williams *et al*, unpublished data, 1999). However, to date, variability has been found in the expression of the proteins involved in apoptosis in oral squamous carcinoma, and further studies are required to determine the pathways involved in apoptosis, which contribute to the balance between cell death and cell proliferation that occurs during tumour growth.

Conclusions

Our understanding of the molecular basis of oral squamous carcinoma has increased rapidly over the past few years. Multiple genetic events that culminate in carcinogenesis include the activation of oncogenes and inactivation of tumour suppressor genes. However, not all genetic events occur in all squamous oral carcinomas and similar genetic alterations may occur at different times in the process of carcinogenisis. This was shown in a recent study in which microsatellite analysis with 52 polymorphic markers at 13 key chromosomal regions implicated in the pathogenesis of head and neck cancers revealed that different pathways lead to cancer, and a variety of allelic imbalances can be detected in individual tumours at all TNM stages.[113]

This may account for the different clinical behaviour of tumours classified as the same TNM stage, and these authors advocate the development of databases summarising the genetic events associated with oral squamous carcinomas.[113] This may increase our understanding of the molecular basis of these lesions and establish whether different cancer subtypes show different growth characteristics. This approach could ultimately lead to appropriate gene therapy.

1 Parkin DM, Laara E, Muir CS. Estimates of the world wide frequency of eighteen major cancers in 1995. *Int J Cancer* 1993;**54**:594–606.
2 Macfarlane G, Boyle P, Evstifeeva T. Rising trends of oral cancer mortality among men worldwide: the return of an old public health problem. *Cancer Causes Control* 1994;**5**: 259–65.
3 Vokes EE, Weichselbaum RR, Lippman SM, *et al.* Head and neck cancer. *N Engl J Med* 1993;**328**:184–94.
4 Jovanic A, Ignaz GH, Pieter J. Secondary respiratory and upper digestive tract cancer following oral squamous cell carcinoma. *Eur J Cancer B Oral Oncol* 1994;**30B**:225–9.
5 Ankathil R, Mathew A, Joseph F. Is oral cancer susceptibility inherited? *Eur J Cancer B Oral Oncol* 1996;**32B**:63–7.
6 Jeffries S, Eeles R, Goldgar D, *et al.* The role of genetic factors in predisposition to squamous cell cancer of the head and neck. *Br J Cancer* 1999;**79**:865–7.
7 Cooper M, Jovanic A, Nauta J. Role of genetic factors in the aetiology of squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 1995;**121**:157–60.
8 Foulkes WD, Brunet J-S, Kowalski LP. Family history is a risk factor for squamous cell carcinoma of the head and neck in Brazil: a case control study. *Int J Cancer* 1995;**63**:769–73.

9 Bongers V, Braakhuis B, Tobi H. The relation between cancer incidence among relatives and the occurrence of multiple primary carcinomas following head and neck cancer. *Cancer Epidemiol Biomarkers Prev 1996;***5/8***:595–8.*
10 Sidransky D. Molecular genetics of head and neck cancer. *Curr Opin Oncol* 1995;**7**:229–33.
11 Todd R, Donoff RB, Wong DTW. The molecular biology of oral cancer: toward a tumour progression model. *J Oral Maxillofac Surg* 1997;**55**:613–23.
12 Bockmuhl U, Wolf G, Schmidt S, *et al.* Genomic alteration associated with malignancy in head and neck cancer. *Head Neck* 1998;**20**:145–51.
13 El-Nagger AK, Hurr K, Batsakis JG, *et al.* Sequential loss of heterozygosity at microsatellite motifs in preinvasive and invasive head and neck squamous carcinoma. *Cancer Res* 1995;**55**:2656–9.
14 Partridge M, Emilion G, Pateromichelakis S, *et al.* Location of candidate tumour suppressor gene loci at chromosomes 3p, 8p and 9p for oral squamous carcinomas. *Int J Cancer* 1999;**84**:318–25.
15 Partridge M, Emilion G, Paternomichelakis S, *et al.* The prognostic significance of allelic imbalance at key chromosomal loci in oral cancer. *Br J Cancer* 1999;**79**:1821–7.
16 Califano J, van der Reit P, Westra W, *et al.* Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996;**56**:2488–92.
17 Emilion G, Langdon JD, Speight P, *et al.* Frequent gene deletions in potentially malignant oral lesions. *Br J Cancer* 1996;**73**:809–13.
18 Partridge M, Emilion G, Paternomichelakis S, *et al.* Allelic imbalance at chromosomal loci implicated in the pathogenesis of oral cancer, cumulative loci and its relationship with progression to cancer. *Eur J Cancer B Oral Oncol* 1998;**34**: 75–83.
19 Sherman JA, Partridge M. Expression of retinoic acid precurcors in normal, dysplastic and malignant oral epithelium. *Br J Oral Maxillofac Surg* 1997;**35**:260–6.
20 Field JK. Oncogenes and tumour suppresser genes in squamous cell carcinoma of the head and neck. *Eur J Cancer B Oral Oncol* 1992;**28**:67–76.
21 Papadimitrakopoulou V, Izzo J, Lippman SM, *et al.* Frequent inactivation of p16 INK 4a in oral premalignant lesions. *Oncogene* 1997;**14**:1799–803.
22 Hunter T, Pines J. Cyclins and cancer II: cyclin d and cdk inhibitors come of age. *Cell* 1994;**74**:573–82.
23 Pande P, Mathur MR, Shukla NK, *et al.* PRb and p16 protein alterations in human oral tumorigenesis. *Eur J Cancer B Oral Oncol* 1998;**34**:396–403.
24 Murray AW. Creative blocks: cell-cycle check points and feedback controls. *Nature* 1992;**359**:599–604.
25 Pines J. Cyclins, CDKs and cancer [review]. *Semin Cancer Biol* 1995;**6**:63–72.
26 Kotelnikov VM, Coon JS, Mundle S, *et al.* Cyclin D1 expression in squamous cell carcinomas of the head and neck and in oral mucosa in relation to proliferation and apoptosis. *Clin Cancer Res* 1997;**3**:95–101.
27 Kushner J, Bradley G, Young B, *et al.* Aberrant expression of cycle A and cyclin B1 proteins in oral cancer. *J Oral Pathol Med* 1999;**28**:77–81.
28 Bishop JM. Molecular themes in oncogenesis. *Cell* 1991;**64**: 235–48.
29 Wong DT, Weller PF, Galli SJ, *et al.* Human eosinophils express transforming growth factor alpha. *J Exp Med* 1990; **172**:673–81.
30 Todd R, Chou MY, Matossian K, *et al.* Cellular sources of transforming growth factor-alpha in human oral cancer. *J Dent Res* 1990;**70**:917–23.
31 Derynck R. The physiology of transforming growth factor-alpha. *Adv Cancer Res* 1992;**58**:27–52.
32 Todd R, Donoff BR, Gertz R, *et al.* TGF-α and EGF receptor mRNAs in human oral cancers. *Carcinogenesis* 1989;**10**: 1553–6.
33 Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of head and neck cancer. *Cancer Res* 1993;**53**: 3579–84.
34 Partridge M, Gullick WJ, Langdon JD, *et al.* Expression of epidermoid growth factor receptor in oral squamous cell carcinoma. *Br J Oral Maxillofac Surg* 1988;**26**:381–9.
35 Scully C. Oncogenes, tumour suppressers and viruses in oral squamous carcinoma. *J Oral Pathol Med* 1993;**22**:337–47.
36 Christensen ME, Therkildsen MH, Hanson BL, *et al.* Immunoreactive transforming growth factor-α and epidermal growth factor in oral squamous cell carcinomas. *J Pathol* 1993;**169**:323–8.
37 Santine J, Formento JL, Frauncoual M, *et al.* Characterisation, quantification and potential clinical value of epidermal growth factor receptor in head and neck squamous carcinomas. *Head Neck* 1991;**13**:132–39.
38 Kutton NAA, Govard Ivan, Bhak NM. Epidermal growth factor receptor (EGFR) in oral squamous cell carcinomas: overexpression, localization and therapeutic implications. *Indian J Dent Res* 1997;**8**:9–18.
39 Cantley LC, Auger KR, Carpenter C, *et al.* Oncogenes and signal transaction. *Cell* 1991;**64**:281–302.
40 McDonald JS, Jones H, Pavelic ZP, *et al.* Immunohistochemical detection of the H-ras, K-ras and N-ras oncogenes in squamous cell carcinoma of the head and neck. *J Oral Pathol Med* 1994;**23**:342–6.
41 Wendell G, Yarborough MD, Shores C, *et al.* Ras mutations and expression in head and neck squamous cell carcinomas. *Laryngoscope* 1994;**104**:1337–47.
42 Kiaris H, Spandidos DA, Jones AS, *et al.* Mutations, expression and genomic instability of the H-ras oncogene in chewing tobacco-related human oral carcinoma in India. *Br J Cancer* 1991;**63**:70–4.
43 Milasin J, Pujic N, Dedovic N, *et al.* High incidence of H-ras mutations in squamous cell carcinoma of lip vermillion. *J Oral Pathol Med* 1994;**23**:298–301.
44 Saranath D, Change Se, Bhotie LT, *et al.* High frequency of mutations in codons 12 and 61 of H-ras proto-oncogene in squamous cell carcinomas of the head and neck. *Br J Cancer* 1991;**63**:573–8.
45 Kuo MYP, Jeng JK, Chiang CP, *et al.* Mutations of Ki-ras oncogene codon 12 in betel quid chewing-related human squamous cell carcinoma in Taiwan. *J Oral Pathol Med* 1994;**23**:70–4.
46 Field JK. The role of oncognes and tumour suppressor genes in the aetiology of oral, head and neck squamous cell carcinoma. *J R Soc Med* 1995;**88**:35P–9P.
47 Spandidos DA, Lamonthe A, Field JK. Multiple transcriptional activation of cellular oncogenes in human head and neck solid tumours. *Anticancer Res* 1985;**5**:221–4.
48 Hooper ML. The role of p53 and Rls-1 genes in cancer development and apoptosis. *J Cell Sci* 1994;**13**:13–17.
49 McConkey DJ, Zhivotosvsky B, Orrenius S. Apoptosis—molecular mechanisms and biochemical implications. *Mol Aspects Med* 1996;**17**:1–110.
50 Williams ME, Gaffey MJ, Weiss LM, *et al.* Chromosome 11q13 amplification in head and squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 1993;**119**:1238–43.
51 Callender T, El-Nagger AK, Lee MS, *et al.* PRAD-1 (CCND)/cyclin D1 oncogene amplification in primary head and neck squamous cell carcinoma. *Cancer* 1994;**74**: 152–8.
52 Yoshida T, Miyagawa K, Odagiri H, *et al.* Genomic sequence of hst, a transforming gene encoding a protein homologous to fibroblast growth factor and the int-2 encoded protein. *Proc Natl Acad Sci U S A* 1987;**84**:7305–9.
53 Myoken Y, Okamoto T, Sato JD, *et al.* Immunocytochemical localisation of fibroblast growth factor-1 (FGF-1) and FGF-2 in oral squamous carcinoma (SCC). *J Oral Pathol Med* 1994;**23**:451–6.
54 Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987; **235**:442–7.
55 Lese CM, Rossie KM, Appel BN, *et al.* Visualisation of int-2 and HST1 amplification in oral squamous cell carcinomas. *Genes Chromosomes Cancer* 1985;**12**:288–95.
56 Somers KD, Cartwright SL, Schechter GL. Amplification of the int-2 gene in human head neck squamous cell carcinomas. *Oncogene* 1990;**5**:915–20.
57 Lee WH. Tumour suppressor genes—the hope [editorial]. *FASEB J* 1993;7:819.
58 Yokota J, Sugimura T. Multiple steps in carcinogenesis involving alterations of multiple tumour suppress genes. *FASEB J* 1993;7:920–5.
59 Vogelstein B, Kinzier KW. The multistep nature of cancer. *Trends Genet* 1993;**9**:138–41.
60 Harper JW, Adamy GR, Wei N, *et al.* The p21 cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993;**75**:805–16.
61 El-Deiry WS, Tokino T, Velculesco VE, *et al.* WAF1, a potential mediator of p53 tumour suppression. *Cell* 1993;**75**:817–25.
62 Datto MB, Li Y, Panus JF, *et al.* Transforming growth factor induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc Natl Acad Sci U S A* 1995;**92**:5545–9.
63 Thomas R, Kaplan L, Reich N, *et al.* Characterization of human p53 antigens employing primate specific monoclonal antibodies. *Virology* 1983;**131**:502–17.
64 Iggo R, Gatter K, Bartek J, *et al.* Increased expression of mutant forms of p53 oncogene in primary lung cancer. *Lancet* 1990;**335**:675–9.
65 Hollstein M, Sidransky D, Volgelstein B, *et al.* p53 mutations in human cancers. *Science* 1991;**253**:49–53.
66 Schantz SP. Basic science advances in head and neck oncology; the past decade. *Semin Surg Oncol* 1995;**11**:272–9.
67 Langdon JD, Partidge M. Expression of the tumour suppressor gene p53 in oral cancer. *Br J Oral Maxillofac Surg* 1992;**30**:214–20.
68 Brennan A, Boyle JO, Koch WM. Association between cigarette smoking and mutation of the p53 gene in squamous cell carcinoma of the head and neck. *N Engl J Med* 1995:**332**:712–17.
69 Greenblatt MS, Bennett WP, Hollstein M, *et al.* Mutations in the p53 tumour supressor gene: clues to cancer aetiology and molecular pathogenesis. *Cancer Res* 1994;**54**:4855–78.
70 Lianes P, Orlow I, Zhang ZF, *et al.* Altered patterns of mdm2 and TP53 expression in human bladder cancer. *J Natl Cancer Inst* 1994;**86**:1325–30.
71 Heinzel PA, Balaram P, Hill B. Mutations and polymorphisms in the p53, p21 and p16 genes in oral carcinomas of Indian betel quid chewers. *Int J Cancer* 1996;**68**:420–3.
72 Agarwal S, Mathur, Shukla NK, *et al.* Expression of cyclin dependent kinase inhibitor p21 waf1/cip1 in premalignant and malignant oral lesions: relationship with p53 status. *Eur J Cancer B Oral Oncol* 1998;**34**:353–60.
73 Ng IOL, Lam KY, Ng M, *et al.* Expression of p21/waf1 in oral squamous cell carcinomas—correlation with p53 and mdm2 and cellular proliferation index. *Eur J Cancer B Oral Oncol* 1999;**35**:63–9.

74 Shiohara M, El-Deiry WS, Wada N, *et al.* Absence of WAF1 mutations in a variety of human malignancies. *Blood* 1994; **84**:3781–4.
75 Deng G, Chen L-C, Schott DR, *et al*. Loss of heterogygosity and p53 gene mutations in breast cancer. *Cancer Res* 1994; **54**:499–505.
76 Brooks JD, Bova GS, Ewing CM, *et al*. An uncertain role for p53 gene alterations in human prostate cancers. *Cancer Res* 1996;**56**:3814–22.
77 Brachmann RK, Vidal M, Boeke JD. Dominant-negative mutations in yeast hit cancer hot spots. *Proc Natl Acad Sci U S A* 1996;**93**:4091–5.
78 Varley JM, Brammar WJ, Lane DP, *et al*. Loss of chromosome 17p 13 sequences and mutation of p53 in human breast carcinomas. *Oncogene* 1991;**6**:413–21.
79 Ahomadegbe JC, Barrois M, Fogel S, *et al.* High incidence of p53 alterations (mutation, deletion, overexpression) in head and neck primary tumours and metastases; absence of correlation with clinical outcome. Frequent protein overexpression in normal epithelium and in early non-invasive lesions. *Oncogene* 1995;**10**:1217–27.
80 Xu L, Chen Y-T, Huvos AG, *et al*. Overexpression of p53 in squamous cell carcinoma of head and neck without apparent gene mutations. *Diagn Mol Pathol* 1994;**3**:83–92
81 Partridge M, Kiguwa S, Emilion G, *et al*. New insights into p53 protein stabilisation in oral squamous cell carcinoma. *Eur J Cancer B Oral Oncol* 1999;**35**:45–55.
82 Midgeley CA, Lane DP. p53 protein stability in tumour cells is not determined by mutation but is dependent on mdm2 binding. *Oncogene* 1997;**15**:1179–89.
83 Haupt Y, Maya R, Kazaz A, *et al*. Mdm2 provides the rapid degradation of p53. *Nature* 1997;**387**:296–9.
84 Lane DP, Hall PA. MDM2—arbiter of p53's destruction. *Trends Biol Sci* 1997;**22**:372–4.
85 Brachman DG, Graves D, Vokes E, *et al*. Occurrence of p53 gene deletions and human papilloma virus infection in human head and neck cancer. *Cancer Res* 1992;**52**:4832–6.
86 Min B-M, Baek JH, Shin KH, *et al*. Inactivation of the p53 gene by either mutation or HPV infection is extremely frequent in human oral squamous carcinoma cell lines. *Eur J Cancer B Oral Oncol* 1994;**30B**:338–45.
87 Todd R, McBride J, Tsuji T, *et al*. Deleted in oral cancer-1 (doc-1) a novel oral tumour suppressor gene. *FASEB J* 1995;**9**:1362–70.
88 Rustgi AK, Dyson N, Hill D, *et al*. The c-myc oncoprotein forms a complex with the product of the retinoblastoma gene. *Cold Spring Harbor Symp Quant Biol* 1991;**56**:163–7.
89 Largey JS, Meltzer SJ, Sauk JJ, *et al*. Loss of heterozygosity involving the APC gene in squamous cell carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1994;**77**:260–3.
90 Huang JS, Chiang CP, Kok S-H. Loss of heterozygosity of APC and MCC genes in oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 1997;**26**:322–6.
91 Behrens J, Vakaet L, Frii R, *et al*. Loss of epithelial differentiation and gain of invasiveness correlates with tyrosine phosphorylation of the E-cadherin/βcatenin complex in cells transformed with a temperature-sensitive V-SRC gene. *J Cell Biol* 1993;**120**:757–66.
92 Bagutti C, Speight PM, Watt FM. Comparison of integrin, cadherin and catenin expressions in squamous cell cancinomas in the oral cavity. *J Pathol* 1998;**186**:8–16.
93 Williams HK, Sanderes DSA, Jankowski JAZ, *et al*. Expression of cadherins and catenins in oral epithelial dysplasia and squamous cell carcinoma. *J Oral Pathol Med* 1998;**27**: 308–17.
94 Becker KF, Atkinson MJ, Reich U, *et al*. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res* 1994;**54**:3845–52.
95 Behrens J, von Knes JP, Kuhl M, *et al.* Functional interaction of β-catenin with the transcription factor LEF-1. *Nature* 1996;**382**:638–42.
96 Morin PJ, Sparks AB, Korinek V, *et al.* Activation of β-catenin—Tcf signalling in colon cancer by mutations in β-catenin or APC. *Science* 1997;**275**:1787–90.
97 Korineck V, Barker N, Morin PJ, *et al.* Constitutive transcriptional activation by a β-catenin–Tcf complex in APC–/– colon carcinoma. *Science* 1997;**275**:1784–7.
98 Li-Kuo S, Vogelstein B, Kinzler KW. Association of the APC tumour suppressor protein with catenins. *Science* 1993;**262**: 1734–7.
99 Smith MEF, Pagnatelli M. The molecular histology of neoplasia: the role of the cadherin/catenin complex. *Histopathology* 1997;**31**:107–11.
100 Garzino-Demo P, Carrozo M, Trusolino L. Altered expression of alpha 6 integrin sub unit in oral squamous cell carcinoma and oral potentially malignant lesions. *Eur J Cancer B Oral Oncol* 1998;**34**: 204–10.
101 Shinohara M, Nakamura S, Sasaki M. Expression of integrins in squamous cell carcinoma of the oral cavity. Correlations with tumour invasion and metastasis. *Am J Clin Pathol* 1999;**111**:75–88.
102 Thomas GJ, Jones J, Speight PM. Integrins and oral cancer. *Eur J Cancer B Oral Oncol* 1997;**33**:381–8.
103 Jones J, Watt FM, Speight PM. Changes in expression of alpha V integrins in oral squamous cell carcinomas. *J Oral Pathol Med* 1997;**26**:63–8.
104 Boyd D. Invasion and metastasis. *Cancer Metastasis Rev* 1996;**15**:77–89.
105 Tryggvason K, Hoyhtya M, Sale T. Proteolytic degradation of extra cellular matrix in tumour invasion. *Biochim Biophys Acta* 1987;**907**:191–217.
106 Flaitz CM, Hicks MJ. Molecular piracy: the viral link to carcinogenesis. *Eur J Cancer B Oral Oncol* 1998;**34**:448–53.
107 Carson DA, Ribeiro JM. Apoptosis and disease. *Lancet* 1993;**341**:1251–4.
108 Bentler B, van Huffel C. Unravelling function of the TNF ligand and receptor families. *Science* 1994;**264**:667–8.
109 Nagata S, Golstein P. The fas death factor. *Science* 1995;**267**:1449–56.
110 Loro LL, Vintermyr OK, Johannessen AC, *et al.* Suppression of Fas receptor and negative correlation of Fas ligand with differentation and apoptosis in oral squamous cell carcinoma. *J Oral Pathol Med* 1999;**28**:82–7.
111 Jordan RCK, Catzavelos GC, Barrett AW, *et al*. Differential expression of bcl-2 and bax in squamous cell carcinoma of the oral cavity. *Eur J Cancer B Oral Oncol* 1996;**32B**:394–400.
112 Ravi D, Ramadas K, Matthew BS, *et al*. De novo programmed cell death in oral cancer. *Histopathology* 1999; **34**:241–9.
113 Partridge M, Emilion G, Falworth M, *et al*. Patient-specific mutation databases for oral cancer. *Int J Cancer* 1999;**84**: 284–92.